NUCLEAR AND CYTOPLASMIC INTERRELATIONS IN THE FERTILIZATION OF THE ASTERIAS EGG

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The existence of a functional relation between nucleus and cytoplasm is generally accepted, but there are relatively few instances in which the relationship can be demonstrated experimentally. Among ova an extreme case is that exhibited by the maturing ovum. Fol (1877, 1879), in his classic work [1] on the maturation and fertilization of the egg of *Asterias glacialis,* was probably the first to associate the maturation of the ovum with the breakdown of the germinal vesicle. In general, the significance of maturation of the ovum has been too closely limited to the elimination of the polar bodies. More attention should be given to what is probably the basic feature of the phenomenon, namely, changes incurred in the cytoplasm through the admixture of nuclear material from the enlarged germinal vesicle of the ovarian egg (cf. maturation cytoplasmique of Delage, 1901; and R. Chambers, 1921). It has been recently proposed (R. Chambers, 1949) that the cytoplasm of the maturing and mature egg be termed *karyocytoplasm.*

There is also to be considered a relationship between the male and female nuclear elements of the fertilized egg and of both elements with the maturing karyocytoplasm. Many observers have ascribed the movements of the male and female pronuclei to their mutual attraction across the intervening cytoplasm of the egg. An early attempt at testing the existence of such an attraction was made by George Lester Kite, a pioneer in microdissection. In a lecture (unpublished) given during the summer of 1915 at the Marine Biological Laboratory, Woods Hole, Dr. Kite described his efforts at interposing the tip of a microneedle as an obstacle between the male and female pronuclei in the transparent egg of Lytechinus, then known as Toxopneustes. As he dramatically stated: "The pesky nuclei insisted in slipping around the obstacle and no efforts, short of destroying the egg, could prevent the nuclei from approaching one another and uniting."

More recently, E. L. Chambers (1939) was able to offer an interpretation in

[1] Fol's 1879 paper is extraordinary for the abundance and accuracy of his extended observations on the living Echinoderm egg. His assumption of extruded cytoplasmic filaments of the Asterias egg which serve to draw the blunt-nosed spermatozoa through the surrounding jelly to the surface of the egg had been largely discredited until fully substantiated many years later not only for Asterias but also for many of the Asteroidea. Even in regard to the quadrille des centres described by Fol in his paper of 1891 and attacked by Wilson and Mathews (1895), Fol had a case. In his 1879 paper (p. 210) Fol remarked that in heavy polyspermy the sperm asters assume identical distances from one another placed with their centers along a theoretical circle. This fits in with the findings of E. L. Chambers (1939) regarding the sperm aster as a growing spherical gelated body. Several sperm asters simultaneously growing in size would assume the positions ascribed to them by Fol. Such symmetrical positions of four sperm asters would explain Fol's quadrille des centres. It was unfortunate that Fol was not able to correct his one wrong hypothesis because of his untimely death soon after publication of his paper.

terms of physical changes in the cytoplasm. He showed that the movements could be ascribed to the growing sperm aster as a gelated body (R. Chambers, 1917), the sperm pronucleus lying in or close to the center of the aster. The progressive increase in size of the aster transfers the sperm pronucleus passively to a central position in the egg, while the egg pronucleus is carried to the sperm pronucleus by centripetal streaming in radial channels converging at the center of the aster. Fol (1879, pp. 105 and 194), who first described the aster, had already presented the idea that the astral radiations are due to streams of centripetal flow.

The normal dissolution of the germinal vesicle of the fully grown oocyte initiates a gradual and prolonged process (R. Chambers, 1921) which converts the somatic cytoplasm of the ovarian egg into the karyocytoplasm of the maturing egg ready for fertilization. The experiments described in this paper, a brief account of which has been published (R. Chambers and E. L. Chambers, 1940), present the matter in detail with evidence concerning hitherto unsuspected causal interrelations between the egg nucleus, the sperm pronucleus, and the egg cytoplasm during and after alteration of the cytoplasm by the spontaneous dissolution of the germinal vesicle. These interrelations constitute, as it were, the performances of a three ring circus in the maturation of the egg.

The experiments stress features which are concerned with the egg and sperm nuclei during their earlier stages before the sperm aster has attained full expression. They are not to be compared with the egg fragmentation studies of Delage (1899), Tennent, Taylor and Whitaker (1929) and Whitaker (1928), all of which were done on fully mature sea urchin eggs and with reconstituted female pronuclei, both polar bodies already having been eliminated.

Material and Methods

The starfish egg is admirably suited for the present study, since, commencing with the germinal vesicle stage, the eggs develop in sea water and insemination can take place at any time.

Fol (1877) had observed that the eggs of *Asterias glacialis* normally are expelled into the sea water with the germinal vesicle still intact. In our work the fully grown germinal vesicle eggs were uniformly obtained by removing the ripe ovaries into finger bowls of sea water, where the eggs were immediately distributed in a large volume of sea water. Most of the work was done during the months of June and July. Only those batches of eggs were used in which over 90 per cent of samples of the eggs matured. All the bisecting operations on the eggs were done under oil and water immersion objectives.

The fragmented eggs and their controls were maintained at a temperature of 16° C. in syracuse watch glasses. The operations and observations were made in hanging drops suspended from a coverslip in the moist chamber of a micromanipulator at room temperature. The eggs were transferred to the moist chamber, and several eggs immediately bisected. This required about three or four minutes. The eggs were then replaced in the watch glasses at 16° C., kept there until a few minutes before appearance of the sperm aster was expected, and then re-transferred to the moist chamber for observation.

The bisections were performed on the eggs at varying intervals after dissolution of the germinal vesicle, some before and others after insemination. The eggs,

suspended from the roof of the moist chamber, were divided by compressing them with the horizontal shaft of a slender microneedle. The vitelline membrane of the unfertilized egg and the enveloping membrane of the fertilized egg are firm enough to remain more or less intact during the bisection. The two egg fragments, which immediately round up and are completely separated, tend to remain together. The cutting was generally done so as to have both fragments of about the same size, one fragment never being smaller than about one half the volume of the other. Such a difference in size had no appreciable effect on the time of appearance of the polar bodies or of the sperm aster. This is in accord with Tennent, Taylor, and Whitaker (1929) who had shown that the cleavage time of egg fragments is independent of size as long as the fragments, when fertilized, undergo segmentation.

In all the experiments, every individual fragment was kept under observation simultaneously with its companion fragment in the same microscopic field. Hence, when a phenomenon was detected in one fragment it could be immediately compared with what might appear in the companion fragment. The time sequences and the phenomena looked for in each individual case were so clear-cut that intervals as short as two minutes were significant. The phenomena observed were the appearance in the granular cytoplasm of a diminutive radiating star which represented the sperm aster, and the elevation of a hyaline nipple on the surface of the egg, the beginning of one or other of the polar bodies.

Bisecting eggs with intact germinal vesicles confirmed the already recognized finding that fragments lacking the germinal vesicle are not fertilizable (Delage, 1901). After normal dissolution of the germinal vesicle, both fragments are capable of being fertilized, one with a diploid (sperm and egg), and the other with a haploid (sperm) nucleus.

The bisection of eggs already inseminated was done at varying times prior to first polar body formation. As was to be expected, only those fragments were capable of further development which contained the sperm pronucleus. Special attention was given to those eggs in which the sperm and egg nuclei were separated, one in each fragment.

Results

The investigation is classified under two general headings. The first deals with observations on the sequence of events in whole eggs, and the second with bisected eggs. In the latter, attention was directed toward the reactions of the male and female nuclei when together and when isolated in the respective fragments of karyocytoplasm.

I. Observations on the Whole Egg

A. The unfertilized egg

The first intimation of the dissolution of the germinal vesicle is the development of an irregular contour of the membrane and a fading from view of the prominent nucleolus. An irregularity in shape of the membrane is not necessarily related to impending dissolution of the germinal vesicle. A mere collapse of the membrane induced by shaking the eggs does not accelerate maturation. The one

visible change which consistently heralds dissolution is the disappearance of the nucleolus. This is followed by disappearance of the nuclear membrane and a diffusion of the nucleolar contents mixed with the hyaline karyoplasm of the nucleus into the granular cytoplasm. Within five to fifteen minutes, the region formerly occupied by the germinal vesicle is filled with cytoplasmic granules indistinguishable from the rest of the egg. In the granular cytoplasm it is possible to detect the diminutive, hyaline egg nucleus which later gives off the polar bodies.

TABLE I

Sequence of events in the maturing unfertilized eggs of Asterias forbesii at 16–18° C.

	Time
From time of deposition in sea water to:	
Disappearance of nucleolus (50% completion)	8′– 19′
Formation of 1st polar body (50% completion)	76′– 90′
Formation of 2nd polar body (50% completion)	105′–119′

Table I gives the approximate times of the three most obvious events during the maturation of the unfertilized egg. The data were obtained from ten separate batches of eggs of at least 100 eggs in each, kept at a temperature of 16–18° C. The variations in the times recorded are due to the different batches. Within a single batch the variations did not exceed two to three minutes. The figures to the left denote the times, within a two minute range, recorded for seven of the batches. The figures to the right are of one batch. The times for the two remaining batches lie in between.

B. The fertilized egg

Table II, with data averaged from records of five batches of eggs, presents an analysis of the effect on the appearance of the first and second polar bodies and of

TABLE II

Effect of insemination on time of 1st and 2nd polar body formation, and of 1st cleavage in eggs of Asterias forbesii, at 16° C.

(1) Eggs inseminated at following intervals of time after deposition in sea water:	(2) Time 50% 1st P.B. formation after deposition in sea water:	(3) Time 50% 2nd P.B. formation after deposition in sea water:	(4) Time 50% 1st cleavage after deposition in sea water:	(5) Time 50% 1st cleavage after 2nd P.B. formation:	(6) Time 50% 1st cleavage after insemination:
—	78.2 (unfertilized)	107.3 (unfertilized)	—	—	—
25′	70.0	98.0	169.0	70.5	144.5
40′	71.5	101.0	172.0	71.0	132.0
50′	74.0	102.5	173.0	70.5	123.0
60′	75.8	104.0	175.0	71.0	115.0
70′	77.5	105.5	178.0	72.0	108.0
80′	—	106.5	184.5	78.0	104.5
90′	—	107.0	193.5	86.5	103.5
100′	—	107.5	203.5	96.5	103.5
130′	—	—	234.0	127.0	104.0

the first cleavage by inseminating the eggs at successive intervals following breakdown of the germinal vesicle. The first column gives the times of insemination. The figures in the second and third columns show that, up to a certain time, the earlier the insemination the more accelerated is the formation of the polar bodies. When the insemination is delayed to and beyond the time of first polar body formation, there is no evidence of acceleration, whereupon, the time of appearance of the second polar body tends to coincide with that of its appearance in the unfertilized egg.

Evidently it is only when the fertilization process is started early that the conversion of the egg nucleus into its pronucleus is accelerated. Later, when fertilization occurs at the time that the polar body formation has been initiated, there is no longer any appreciable accelerating action.

A consideration of the cleavage times, presented in the fourth, fifth and sixth columns, brings out several significant features. From the fourth column it can be seen, as is to be expected, that cleavage time corresponds with the time the eggs are deposited in sea water. However, during the earlier stages, up to some time before first polar body formation (after 60 minutes), the lapse is not as great as during the later stages (cf. Fig. 1). This is brought out more clearly from the figures in the fifth column which give the times between those of first cleavage and of second polar body formation. They indicate that the time interval, irrespective of insemination time, is constant until about the time when the first polar body is being initiated. After this the cleavage time becomes directly proportional to the insemination time.

The figures in the sixth column give the times between insemination and first cleavage. They show that the earlier the insemination up to the time when the first polar body is initiated (about 70 minutes), the longer is the time which elapses before cleavage occurs. After 70 minutes the time between insemination and cleavage becomes constant.

These analyses indicate that the rate at which the fertilization events proceed depends upon the cytoplasmic maturation which is completed at about the time of first polar body formation. Prior to this, it would seem that the immature state of the karyocytoplasm has a delaying effect on the development of the sperm and its accompanying events. Upon initiation of first polar body formation, the maturation of the karyocytoplasm is complete, whereupon the development of the sperm from the time of its entry proceeds without delay and cleavage occurs within a constant period of time.

A graphic presentation of Table II is given in Figure 1. The abscissae represent the times of insemination; the ordinates, the times when the various events occur. Concerning the unfertilized egg, the two vertical dotted lines and the two horizontal dotted lines intercept the X and Y axes respectively at the times when the first polar body forms (average of 78.2 minutes) and when the second polar body forms (average of 108.2 minutes).

Concerning the fertilized egg, the three solid curves represent the times for the formation, respectively, of the first and of the second polar bodies, and of the first cleavage in eggs inseminated at different intervals after germinal vesicle breakdown. The curves for the first and second polar body formation are parallel throughout and their upward slopes represent the acceleration due to insemination.

It is to be noted that when the insemination occurs at 78 minutes (time of first polar body formation) or later, the time of second polar body formation remains the same as that of the unfertilized egg.

Let us now consider the dotted dash curve which represents the time of first appearance of the sperm aster and which was calculated from data obtained on about 100 eggs observed with an oil immersion objective. The sperm aster never appears until after the second polar body, no matter how early the eggs have been inseminated (the earliest recorded being at 25 minutes). During these earlier

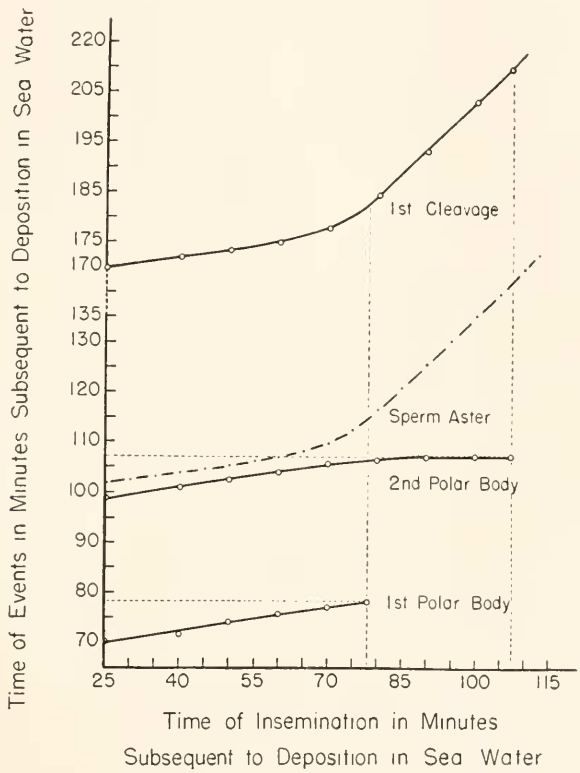

FIGURE 1. Relation of time of insemination to time of appearance of first P.B., second P.B., and first cleavage (solid lines), and of sperm aster (dotted dash line). Faint dotted horizontal lines represent times of appearance in unfertilized eggs of first and second P.B. The observations were made on eggs of *Asterias forbesii* at 16° C.

stages of the developing egg, it appears at two to three minutes after the second polar body has been given off. In later stages, viz., after 70 minutes, this interval becomes progressively greater. It is to be noted that the interval between the time of appearance of the sperm aster and the time of first cleavage is always just about 68 minutes, whether the time of insemination is early or late. This is the normal time for the events following aster appearance. The length of the interval between the time of insemination and the time of sperm aster appearance varies, depending upon the state of maturation of the karyocytoplasm. It becomes con-

stant only when the egg is fully mature, which also coincides with the time of first polar body formation. Thus, when the eggs are inseminated as early as 25 minutes after being placed in sea water, a period of about 78 minutes must elapse before the sperm aster appears. When the egg is inseminated during or after the first polar body formation, the interval between insemination and appearance of the sperm aster is found to be constantly about 35 minutes.

Insemination after formation of the first polar body results, as Lillie (1915) has shown, in a tendency toward a decline of fertilizability and of subsequent development. Abnormalities become pronounced when eggs are inseminated 30–60 minutes after the formation of the second polar body.

To summarize: there is a period of progressive ripening of the karyocytoplasm. Optimum ripening is heralded by the development of the polar asters and the initiation of the sperm pronucleus to form its aster. After this period there is a decline in the proper functional interrelations between the sperm and karyocytoplasm. The decline is made evident by the fact that sperm entry, subsequent to second polar body formation, results in an increasing abnormality of cleavage.

II. Observations on Bisected Eggs

A. Unfertilized eggs bisected and the fragments immediately inseminated

1. **Early bisections up to about ten minutes before first polar body formation.** Thirty pairs of fragments were studied. The cutting was done at 25, 40 and 60 minutes after deposition of the eggs in sea water (ca. 10, 25 and 45 minutes respectively after the germinal vesicle had disappeared). Each pair of fragments was then inseminated immediately. Figure 2 is representative of all the cases. The sperm aster in the non-egg-nucleated fragment appeared earlier than in the egg-nucleated fragment. Its time of appearance was always *after* the companion fragment had formed its first polar body and two to three minutes *before* the formation of the second polar body. On the other hand, in the egg-nucleated fragment, the sperm aster never appeared until two to three minutes after the second polar body had been formed. This difference between the two fragments was reflected in the earlier cleavage of the haploid fragment.

2. **Late bisections immediately before and during first polar body formation.** Twenty pairs of fragments were studied. In all of them the sperm aster appeared simultaneously at about two to three minutes after formation of the second polar body in both non-egg-nucleated and egg-nucleated fragments of each pair. The cleavage time of both fragments was simultaneous.

B. Eggs fertilized early and fragmented at varying times until shortly after first polar body formation

The eggs were inseminated 25 minutes after deposition in sea water, that is, shortly after dissolution of the germinal vesicle. In many of the bisected eggs both male and female nuclei lay in the same fragment. These double nucleated fragments, regardless of the time of cutting, behaved exactly like the whole eggs in regard to the time of polar body formation, appearance of the sperm aster and subsequent cleavage. Attention was devoted to the few fragments in which the

sectioning had separated the sperm from the egg nucleus. Four fragments were of eggs cut at 35 minutes; three at 50 minutes, and six at 74 minutes after deposition in sea water.

The results are shown in Figure 3. In the eggs (A_1) cut 35 minutes after deposition in sea water, the sperm aster (A_2) appeared after the first polar body of the companion fragment and two to three minutes before the second polar body. In an egg (B_1) cut at 50 minutes, the sperm aster (B_2) appeared simultaneously with the second polar body of the companion fragment. In an egg (C_1) cut at 74 minutes, the sperm aster (C_2) appeared after the second polar body in the companion fragment. D represents the first cleavage stage, at 170 minutes, of the eggs, A, B, and C. Cleavage occurred, as is to be expected, only in the fragment containing the sperm pronucleus. The egg nucleus in the other fragment produced the first and second polar bodies at the same rate as that of fertilized control whole eggs and, finally, moved to a central position in the fragment, where, as the female pronucleus, it enlarged somewhat but otherwise remained quiescent. The time of appearance of the polar bodies was thus seen to be the same, irrespective of when the sperm pronucleus had been separated from the egg nucleus by the cutting process. Evidently neither a brief nor a long sojourn of the sperm pronucleus in cytoplasmic continuity with the egg nucleus affects the hastening which the fertilization process induces in the formation of the polar bodies.

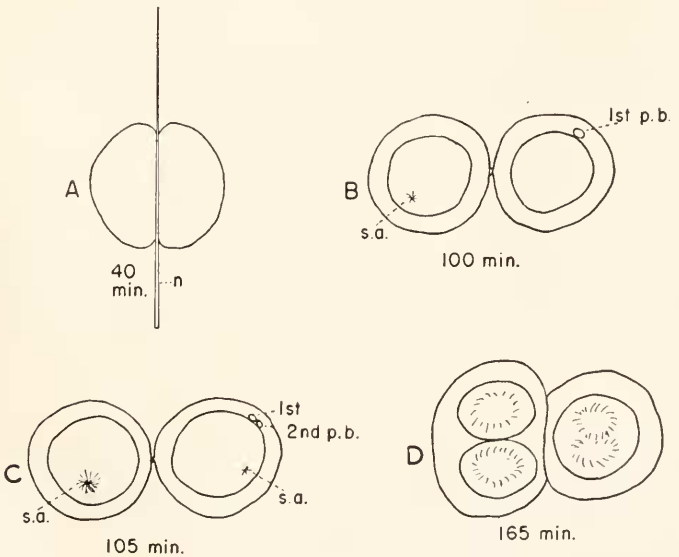

FIGURE 2. Asterias egg bisected 40 minutes after deposition in sea water, both fragments inseminated simultaneously.

A. At 40 minutes. Cutting of unfertilized egg with microneedle, *n*.

B. At 100 minutes. Both fragments with fertilization membranes. Haploid fragment with sperm aster, *s.a.* Diploid fragment with first P.B. which had formed 25 minutes earlier.

C. At 105 minutes. Haploid fragment with considerably enlarged sperm aster. Diploid fragment with beginning sperm aster and second P.B. which had formed two minutes earlier.

D. At 165 minutes. Haploid fragment just after completion of first cleavage. Diploid fragment still in amphiaster stage.

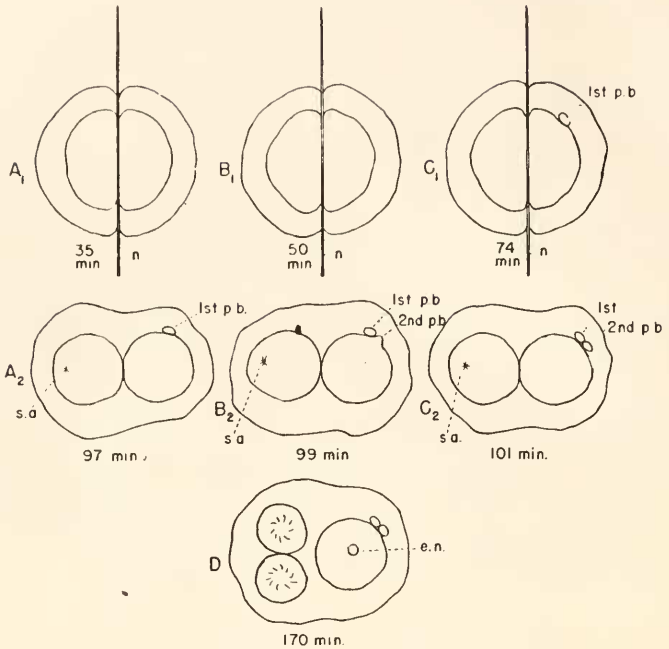

FIGURE 3. Three eggs inseminated 25 minutes after deposition in sea water and then bisected at different times so as to have sperm pronucleus in one fragment and egg nucleus in the other.

A. Cut at 35 minutes (10 minutes after insemination), A_1. At 97 minutes, A_2. Beginning sperm aster, *s.a.*, appears in one fragment and first P.B. in companion fragment.

B. Cut at 50 minutes (25 minutes after insemination), B_1. At 99 minutes, B_2. Beginning sperm aster appears in one fragment and beginning second P.B. in companion fragment.

C. Cut at 74 minutes, just after the first P.B. has formed, C_1. At 101 minutes, C_2. Beginning sperm aster appears in one fragment and completed P.B. in companion fragment.

D. Condition of all three bisected eggs at 170 minutes. Sperm-haploid fragment has cleaved, while egg nucleus, *e.n.*, of companion fragment has taken a central position and remained inactive.

DISCUSSION

The results presented in this paper stress two major features concerning the events after the material of the germinal vesicle has mixed with the cytoplasm of the egg. One deals with the maturation of the karyocytoplasm; the other, with the fertilization process of the male and female nuclear elements in their relations to the maturing karyocytoplasm.

Delage (1899) had already surmised that the dissolution of the germinal vesicle is essential to maturation and fertilizability of the sea urchin egg, and confirmed it from his merogonic experiments (1901) on the immature eggs of *Asterias glacialis*.

In our experiments with *Asterias forbesii* the disappearance of the germinal vesicle and the mixing of its hyaline fluid with the granular cytoplasm lasts about 10–15 minutes. The resulting karyocytoplasm contains the definitive egg nucleus.

Any viable fragment of this karyocytoplasm is fertilizable. The significant feature is that the karyocytoplasm must undergo a protracted maturing process. The prime evidence for completed karyocytoplasmic maturation is the appearance of asters initiated in the egg either by the egg nucleus in forming the polar bodies, or by the spermatozoon in forming its sperm aster.

It is of interest to note that when the fertilization process is initiated in eggs with still maturing karyocytoplasm, the activity of the egg nucleus starts *earlier* than it would if the egg were unfertilized.[2] On the other hand, the activity of the sperm pronucleus in forming its aster starts *later* when it is associated with the egg nucleus than it does when it is isolated in a separate body of karyocytoplasm. This indicates that the fertilization process accelerates the egg nucleus to polar body formation, while the presence of the egg nucleus delays the formation of the sperm aster. As an example of this, let us consider the situation in which an egg nucleus and a sperm pronucleus are lying together in karyocytoplasm which is still maturing. Upon completed maturation of the karyocytoplasm, there is initiated around either the male or the female nucleus a localized, centripetal cytoplasmic streaming which becomes evident to the eye as asters. The first asters to appear are those of the polar spindles of the egg nucleus. Not until the second polar body has been eliminated is there any sign of cytoplasmic streaming centered about the sperm pronucleus for the formation of the sperm aster. The course of these phenomena is of phylogenetic interest, viz., the fact that it is the egg nucleus rather than the sperm around which the radial streaming first occurs.

In the development of the sex elements, the last step taken by the fully grown primary oocyte is to undergo two successive cleavages (equational and reductional). In the early history of sex the resulting four egg cells may be equal in size or, in accordance with later evolutionary changes, they may be unequal, viz., the typical egg and its three polar bodies. In either event, growth of the mother cell, followed by two successive nuclear mitoses, has been repeated presumably over countless periods of time before the male sex cell came into being. This would establish a condition such that the maturation of the karyocytoplasm tends to lead directly to the formation of the two polar bodies. The sperm in the egg is a relatively late comer in evolution so that reactions concerned with it should come after, with the development of the sperm aster and eventually the amphiaster of the first cleavage spindle of the fertilized egg. This might be regarded as a case of evolutionary memory, colloidal or otherwise.

When the sperm pronucleus is isolated in a non-egg-nucleated fragment of an egg with karyocytoplasm which has not yet become mature, maturation leads to cytoplasmic streaming and aster formation about the sperm pronucleus. There is no egg nucleus to assert priority, and the result is that the sperm aster appears before it otherwise would.

When insemination occurs after the karyocytoplasm has completed its maturation, the conditions which now exist do not call for an interplay of the reactions described above. In a completely mature egg, the lapse of 30 to 35 minutes be-

[2] Recently, Lovelace (1947) was able by artificial means to accelerate the penetration of the spermatozoan in the Nereis egg. She found that this induced earlier formation of the polar bodies than would have been the case if the sperm had penetrated later. Fol (1879, pp. 117 and 335) had already noted that for the Asterias egg, polar body formation is accelerated by early insemination of the egg.

tween sperm entry and appearance of the sperm aster is just about the time between the initiation of the first and completion of the second polar body. Therefore, if sperm entry occurs at the earliest moment of completed karyocytoplasmic maturation, i.e., just prior to the formation of the first polar body, the astral streaming of the polar body spindles will have been completed before the sperm aster begins to be appreciable.

An indication of the necessity for proper time relations between the formation of the sperm aster and that of the polar body asters is given in extremely interesting experiments performed years ago by A. Brachet (1922). Brachet discovered a means of disturbing these time relations, and by doing so secured abnormal astral configurations. Brachet found that the immature eggs of Paracentrotus, on being removed from the ovary, could be stopped at various stages of their maturation by plunging the eggs into sea water. In the sea water these eggs readily became polyspermic, and the sperm which had entered continued to develop and formed sperm asters. These asters either remained small or grew to larger dimensions according to the stage of the eggs they were in. The stages of special interest in this discussion were those of the eggs possessing egg nuclear polar spindles and their asters. Sperm asters present at the same time became intermingled with them and formed abnormalities such as tripolar mitoses, etc. Fol was also able to observe similar discrepancies in polyspermic Asterias eggs. In the event that several sperm asters appeared while the chromosomal vesicles of the egg nucleus in mitosis were still infused, Fol noted that one or more of the vesicles became incorporated in the sperm asters, thus upsetting the normal course of events.

The avoidance of such a phenomenon is ensured in the Asterias egg, which normally matures in sea water and which is fertilizable at any stage during its maturation. In monospermic eggs an appropriate time-spacing between the male and female nuclear events is occasioned by the following: On the one hand, the formation of the egg-nuclear polar bodies is accelerated by the fertilization process, while on the other, the appearance of the sperm aster is delayed by the presence of the egg nucleus. The two features combine to separate in time the formation of the polar bodies from the formation of the sperm aster. The result is that in the normal course of development, the cytoplasmic streaming, involved in the formation of the polar body asters, reaches completion before the initiation of the streaming associated with the growing sperm aster. It appears, therefore, that the peculiar interrelations between karyocytoplasm, egg, and sperm nuclei are of service in preventing a possible interference between the reactions concerned in polar body formation and those concerned with preparation of the fertilized egg for its first cleavage.

Summary

Full-sized germinal vesicle oocytes of *Asterias forbesii* undergo normal maturation in sea water. At 16° C. the first polar bodies are formed in about 80 minutes, and the second, in 108 minutes. The eggs are sperm-fertilizable from the time of germinal vesicle breakdown until some time after elimination of the second polar bodies. Fol (1879, p. 204) indicated that the optimum time for insemination is after germinal vesicle breakdown up to the first polar body formation. In accordance with Fol, the earliest period for the sperm aster to appear was found to be

always a few minutes after the formation of the second polar body. A feature to be stressed is the progressive change of the karyocytoplasm induced by the mixing of the contents of the germinal vesicle with the cytoplasm during maturation.

Maturation of the karyocytoplasm

1. When eggs are inseminated two to three minutes before first polar body formation or later, the time for the sperm aster to appear in the living egg is about 35 minutes at 16° C.

When the eggs are inseminated at any time prior to the above, the time taken for the sperm aster to appear is equal to 35 minutes plus the interval between the time of insemination and the time of initiation of the first polar body.

Evidently, therefore, the time of appearance of the sperm aster is a function of the maturation of the karyocytoplasm. The maturation begins at the time of germinal vesicle breakdown and reaches completion two to three minutes prior to formation of the first polar body. In a fully mature karyocytoplasm the interval between sperm entry and the appearance of the sperm aster is constant.

The egg nucleus

2. Sperm-fertilization of whole eggs or of egg-nucleated fragments accelerates the egg nucleus in the formation of its polar bodies. The earlier the insemination the greater is the acceleration.

3. The effect of the fertilization process in accelerating polar body formation persists after removal of the sperm pronucleus. This was ascertained by removing the sperm pronucleus, through bisection, at several intervals of time, the earliest being ten minutes after insemination.

In other words, once given the impetus the egg nucleus maintains its hastened progress independently of the presence of the sperm pronucleus.

The sperm aster

4. In eggs bisected while undergoing maturation and then inseminated, the sperm aster appears earlier in the fragment lacking the egg nucleus than in the egg-nucleated fragment.

In eggs fertilized while undergoing maturation and then bisected at different times, the sooner the sperm pronucleus has been isolated from the egg nucleus, the earlier the sperm aster appears.

In other words, the presence of the egg nucleus has a delaying action on the development of the sperm aster. However, the earlier the egg nucleus has been removed through bisection of the egg, the less is the delaying action.

General Conclusion

There is a close interrelation between (a) the fertilization process, (b) the ripening of the karyocytoplasm, (c) the development of the sperm pronucleus, and (d) the activity of the egg nucleus in forming its polar bodies. The fertilization process, by hastening the maturation of the karyocytoplasm, accelerates the activity of the egg nucleus in forming its polar bodies. On the other hand, the egg nucleus

exerts a lag effect on that feature of the maturation of the karyocytoplasm which is concerned with the development of the sperm aster. The net result is the attainment of an adequate spacing between the times of the cytoplasmic streaming activities concerned with polar body formation and those concerned with the development of the sperm aster. This permits normal development of Asterias eggs fertilized at any time during their maturation.

LITERATURE CITED

Brachet, Albert, 1922. Recherches sur la fécondation prématurée de l'oeuf d'oursin. *Arch. de Biol.*, **32**: 205.

Chambers, E. L., 1939. The movement of the egg nucleus in relation to the sperm aster in the echinoderm egg. *Jour. Exp. Biol.*, **16**: 409.

Chambers, R., 1917. Microdissection studies. II. The cell aster. A reversible gelation phenomenon. *Jour. Exp. Zool.*, **23**: 483.

Chambers, R., 1921. Microdissection studies. III. Some problems in the maturation and fertilization of the echinoderm egg. *Biol. Bull.*, **41**: 318.

Chambers, R. and E. L. Chambers, 1940. Interrelations between egg nucleus, sperm nucleus and cytoplasm of the Asterias egg. *Biol. Bull.*, **79**: 340.

Chambers, R., 1949. Micrurgical studies on the kinetics of cell division. *Annals N. Y. Acad. Sci.* (In press.)

Delage, Yves, 1899. Études sur la mérogonie. *Arch. de Zool. exp. et gen.*, 3e série, **7**: 383.

Delage, Yves, 1901. Études expérimentales sur la maturation cytoplasmique et sur la parthénogenèse artificielle chez les échinodermes. *Arch. de Zool. exp. et gen.*, 3e série, **9**: 285.

Fol, Hermann, 1877. Sur le commencement de l'hénogénie chez divers animaux. *Arch. de Zool. exp. et gen.*, **6**: 145.

Fol, Hermann, 1879. Recherches sur la fécondation, et le commencement de l'hénogénie chez divers animaux. *Mém. de la Société Physique et Hist. nat.* (Genève), **26**: 89.

Fol, Hermann, 1891. Le quadrille des centres, un épisode nouveau dans l'histoire de la fécondation. *Arch. des sci. phys. et nat.*, 3e série, **25**: 393–420.

Lillie, R. S., 1915. Momentary elevation of temperature as a means of producing artificial parthenogenesis and the conditions of its action. *Jour. Exp. Zool.*, **5**: 375.

Lovelace, Roberta, 1947. Precocious sperm entrance. *Anat. Rec.*, **99**: 655.

Tennent, D. H., C. V. Taylor, and D. M. Whitaker, 1929. An investigation on an organization in a sea urchin egg. *Carnegie Inst. of Wash. Publ.* **391**: 1.

Whitaker, D. M., 1928. Localization in the starfish egg and fusion of blastulae from egg fragments. *Physiol. Zool.*, **1**: 55.

Wilson, E. B. and Albert P. Mathews, 1895. Maturation, fertilization, and polarity in the Echinoderm egg. New light on the "quadrille of the centers." *Jour. Morph.*, **10**: 319–342.